UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB - 1 2001

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of a Subchronic Dermal Toxicology Study with 1,2 Benzenedicarboxaldehyde

EPA Reg. No.:010352-00051
1,2 Benzenedicarboxaldehyde
DP Barcode: D270541
PC Code: 129017
Case: 060726
Case Type: Registration

From: S. L. Malish, Ph.D., Toxicologist, Team One, S.L. Malish 1/5/2001
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)

To: Marshall Swindell, PM 33
Karen Leavy-Munk, PM Team Reviewer
Regulatory Management Branch II
Antimicrobials Division (7510C)

Thru: Winston Dang, Team Leader, Team One, 1/26/2001
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)

and

Norman Cook, Chief, RASSB/AD 02/01/2001

Sponsor: Union Carbide Corporation, Danbury, CT

FORMULATION FROM LABEL:

Active Ingredient:	% by weight
1,2 Benzenedicarboxaldehyde	99.8

Synonym: Ortho-phthalaldehyde

Tradename: **UCARCIDE® P200** Antimicrobial

Uses: The commercial product, **UCARCIDE® P200**, containing 1,2 Benzenedicarboxaldehyde (99.6% a.i.) is for the formulation of industrial microbiocides and antimicrobial preservatives.

ACTION REQUESTED:

Toxicology Review of a 9-Day Repeated Skin Contact Study [MRID 452510-01] of the commercial product, **UCARCIDE® P200** Antimicrobial

RECOMMENDATIONS:

This study is classified as **Not-Acceptable**.

The executive summary is noted below. A completed DER is attached.

EXECUTIVE SUMMARY: In a repeated dermal toxicity study (MRID 452510-01) in the rat, 1,2 Benzenedicarboxaldehyde (Ortho-Phthalaldehyde) [99.6% a.i.] was administered to the skin of Sprague-Dawley CD® rats at dose levels of 0, 4, 10 or 20 mg/kg /day for 5 consecutive weekdays, under an occlusive dressing, for a 6 hour duration, after which the skin was wiped clean of excess material. No test/control material was applied on the following 2 days. Dosing was then continued for 4 more days [for a total of 9 days of dosing during the first 11 days of the study]. Control animals received mineral oil at the same volume as the high dose animals. The treatment period was followed by a 4 week [29 day] recovery period.

Physical observations, skin evaluations, body weight, food and water consumption, neurobehavioral evaluations, hematology, clinical chemistry and urinalysis were performed on all animals at selected intervals during the treatment period and/or recovery period.

At the end of the treatment period (day 11), 40 animals (10/sex/group) from all dose levels were sacrificed. In the control and high dose level animals, selected organs were weighed, organ weight ratios calculated and gross and histopathology were conducted. At the end of the recovery period, the remaining animals (5/sex/dose group) in the control and high dose groups were sacrificed for gross pathology including organ weight and ratios.

No test material related deaths occurred. All animals were free of significant pharmacological and toxicological signs throughout the study. The neurobehavorial screen responses were not remarkable compared to the controls. Mean food consumption, water consumption, mean clinical chemistry, mean urinary parameters, and organ weights and ratios an gross and histopathology [except skin] were not remarkable compared to the respective control values.

Mean absolute body weight decreases (<10%) in the high dose treated animals (♂/♀) from days 1 to 11, and the recovery groups from days 15 to 29 were not remarkable. Mean body weight changes showed a 40% decrease in the high dose males and 20% in the intermediate and high dose females from days 1 to 11. The recovery phase [to day 29] also showed decreases in the high dose level males [28%], but not the females vs. the controls.

In the high dose males, the mean white blood cell count exhibited a dose related increase compared to the control at the end of the dosing phase which correlated with an increase [2.5x, $p<0.01$] in the mean absolute neutrophil value. Albeit, all values were within the historical normal range for this strain and age of rat. No changes were seen at the end of the recovery period in either the mean WBC or the mean absolute neutrophil count. In both males and females, the mean hematology values of the remaining treatment groups [♂: low and mid dose; ♀: all dose levels] were comparable to the respective control groups.

The test material produced skin lesions in the mid and high dose animals of both sexes at the application site as evidenced by focal areas of discoloration and/or scabs. The skin also showed, moderate erythema, edema, eschar and/or tissue damage. Histological changes consisted of mild to moderate acanthosis and hyperkeratosis of the epidermis, exudate formation and focal epidermal ulcerations. Most affected animals had accompanying mild, acute dermatitis. Recovery animals, under gross examination, were not remarkable by Day 25.

The **systemic LOAEL** in males is 20 mg/kg/day and is based on a

decrease in mean body weight gain and decrease in the mean WBC, specifically the absolute mean neutrophil count. The **systemic LOAEL** in females is 10 mg/kg/day and is based on a decrease in body weight gain. Body weight gain change resolved in the females but not in the males during the recovery period. The **systemic NOAEL** in males is 10 mg/kg/day and in females 4 mg/kg/day.

The **dermal LOAEL** [♂/♀] is 10 mg/kg/day based on skin irritation; the **dermal NOAEL** is 4 mg/kg/day.

This non-guideline study is classified as **Not-acceptable**, not upgradable and does not satisfy the requirement for FIFRA Test Guideline [§ 82-2] for a Repeated Dose Dermal Toxicity Study in the rat. The study does not meet the minimal requirements of a 21 day dosing period as recommended in the guidelines.

EPA Reviewer: Steven L. Malish, Ph.D.
Team 1, RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Jonathan Chen, Ph.D. Team 3,
RASSB/Antimicrobials Division (7510C)

S. L. Malish 1/29/2001
Jonathan Chen 1/23/2001

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose Dermal Toxicity Study/Rat [§ 82-3, 870.3200]

DP BARCODE: D270541
SUBMISSION CODE: S588267
P.C. CODE: 129017

TEST MATERIAL (PURITY): 1,2 Benzenedicarboxaldehyde [99.6% a.i.]

SYNONYMS: Ortho-phthalaldehyde, UCARCIDE® P200 Antimicrobial

CITATION: Blaszcak, D.L. (2000). 9-Day Repeated Skin Contact Study with Ortho-Phthalaldehyde on the Rat. Huntingdon Life Sciences, East Millstone, NJ. Project ID 96-2457. MRID 452510-01. May 11, 2000. Unpublished.

SPONSOR: Union Carbide Corporation, Danbury, CT

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This non-guideline study is classified as **Not-acceptable**, not upgradable and does not satisfy the requirement for FIFRA Test Guideline [§ 82-2] for a Repeated Dose Dermal Toxicity Study in the rat. The study does not meet the minimal requirements of a 21 day dosing period as recommended in the guidelines.

COMPLIANCE: GLP and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Ortho-Phthalaldehyde (OPA)

Description:	Yellow crystals
Lot #:	TGH0002
Purity:	99.6% a.i.
Date Received:	April 12, 1996
Expiration Date:	March 1997
CAS #	643-79-8

2. Vehicle/Control: Mineral Oil, Mallinckrodt Baker, Inc.

3. Test Animals

Species:	Rat
Strain:	Sprague-Dawley derived [Crl:CD® BR]
Group:	4 groups of 10 or 15 animals/sex
Age:	♂: 7 weeks; ♀: 9 weeks at initiation of treatment
Weight:	♂: 236 gm(218-253); ♀: 225 gm(196-248) at initiation of treatment
Source:	Charles River Breeding Laboratory, Inc. (Kingston, NY)
Housing:	Animals were individually housed in elevated stainless steel wire mesh cages

Diet: Purina Certified Rodent Chow (5002), *ad libitum*
Water: *ad libitum*, by automatic watering system
Environmental:
Temperature: 64 to 79° F
Humidity: 26 to 66%
Air changes: not provided
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In Life Dates

Start: Oct. 28, 1996; End: Nov. 8, 1996, Recovery Animal Necropsy: Dec. 6, 1996

2. Animal Assignment

Animals were assigned to dose groups as indicated in Table 1. Assignment was based on body weight.

Table 1. Animal Assignment[a]

			Number of Animals										
					Clinical Laboratory Studies			Necropsy				Microscopic Pathology	
Dose	Conc.	Dose Vol.	Initial		Day 11 and 12		Week 6	Day 12		Week 6			
mg/kg	mg/ml	ml/kg	♂	♀	♂	♀	♂	♀	♀	♂	♀	♂	♀
0 b	0	4.0	15c	15c	15	15	5	5	5	5	5	10	10
4	1.0	4.0	10	10	10	10	-	-	-	-	-	1d	1d
10	2.5	4.0	10	10	10	10	-	-	-	-	-	3d	2d
20	5.0	4.0	15c	15c	15	15	5	5	5	5	5	10	10

a Adapted from p. 16 [MRID 452510-01].
b Control animals received mineral oil at the same dose as the test animals.
c Five animals/sex were recovery animals.
d Macroscopic lesions were examined from the low and mid dose animals.

B. METHODS

Pilot Study

A pilot study was performed on 3 animals/sex at dose levels of 0, 10, 20 and 30 mg/kg using the procedures similar to that found in the main study. The compound was administered for 5 days. No neurobehavorial tests or gross or histopathology was performed. No pharmacological or toxicological effects were observed except for skin irritation and black staining of the skin. On the basis

of this evaluation, doses for the 9-day study were determined.

1. Analytical

Concentration, homogeneity, and stability were not available in this report.

2. Preparation of dosing mixtures

The test material was ground with a mortar and pestle. The vehicle was then added to an appropriate amount of the test material to form a paste. An appropriate amount of vehicle was then added to the and the mixture was homogenized and then stirred with a stir bar and plate. The control material was stirred with a stir bar ans plate.

3. Dose application

Doses were calculated from the most recent body weight. The test or control material was applied directly onto the exposed skin of the back and sides of the animal, taking care to spread the material evenly over the entire area or as much of the area as can be reasonably be covered. A layer of 8-ply gauze was placed over this site. Animals were dosed for 5 consecutive days A piece of impervious plastic was then placed over the gauze and secured with Elastoplast®. Following 6 hours of exposure, the wrappings were removed and the test site wiped free of test or control material. The animals were allowed to recover for 29 days.

4. Observations

All animals were observed pretest, once prior to each daily dose and twice daily for toxicity and pharmacological effects. Detailed physical examinations for signs of local or systemic toxicity, pharmacological effects and palpation of masses. Recovery animals were examined weekly during the recovery period.

(a) dermal observations

The test sites were scored for dermal irritation prior to study initiation and prior to each daily dose. All animals were also evaluated on Day 12. Recovery animals were evaluated weekly during the recovery period.

5. Body weight

Body weights for all animals were recorded twice pretest and on study days 1, 3, 5, 8 and 11. Recovery animals were also weighed

on Day 15 and then weekly during the recovery period.

6. Food and water consumption

Food and water consumption were measured (weighed) during the week prior to treatment initiation and twice weekly during the

treatment period. Measurements were obtained on study days 3, 5, 8 and 11 and weekly during the recovery period.

7. Neurobehavioral evaluation

This evaluation was preformed pretest once after the fifth dose and again just prior to sacrifice. Recovery animals were evaluated on Day 12. See Appendix 1 for measured parameters.

8. Clinical pathology

Blood was obtained from lightly anaesthetized (CO_2/O_2) animals via puncture of the orbital sinus (retobulbar venous plexus). Rats were fasted overnight prior to blood collections.

(a) hematology

The parameters examined were checked (x) in Table 2 below.

Table 2. Hematology*

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc. (MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular. volume (MCV)
x	Erythrocyte morphology	x	Reticulocyte count
x	Platelet count*		
	Blood clotting measurements*		
x	(Activated partial Thromboplastin time)		
	(Thromboplastin time)		
x	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

(b) clinical chemistry

The parameters examined were checked (x) in Table 3 below.

Table 3. Clinical Chemistry*

	ELECTROLYTES		OTHER
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Direct bilirubin
		x	Indirect bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)*
	Cholinesterase (ChE)	x	Triglycerides
x	Creatine phosphokinase		Serum protein electrophoresis
x	Lactic acid dehydrogenase (LDH)		
x	Serum alanine aminotransferase [ALT]*		
x	Serum aspartate aminotransferase [AST]*		
x	Gamma glutamyl transferase (GGT)		
x	Glutamate dehydrogenase		
x	Sorbitol dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

(c) urinalysis

Urinalysis was performed for approximately half of the animals on freshly voided urine. The parameters examined were checked (x) in Table 4 below.

For the remaining animals, 16 hour urine was evaluated. The parameters examined only at this time period were checked [xx] in Table 4 below.

Table 4. Urinalysis*

x	Appearance	x	Glucose
xx	Volume	x	Ketones
x	Specific gravity	x	Bilirubin
x	pH	x	Blood
x	Sediment (microscopic)		Nitrate
x	Protein	x	Urobilinogen
xx	creatinine		
xx	osmolality		
xx	N-acetyl-β-D-glucosaminidase		

* Not required for subchronic studies according to OPPTS Health Effects Test Guidelines.

9. Pathology

Sacrifice was performed by carbon dioxide inhalation followed by exsanguination. Animals were fasted prior to scheduled sacrifices.

(a) Gross pathology and organ weights

Eighty (80) animals (10/sex/group) were sacrificed in the main group while twenty (20) animals (5/sex/groups) in the control and high dose) were sacrificed in the recovery group.

Examination included examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

The adrenal glands, brain, kidneys, liver, ovaries and the testes with epididymides were weighed. Paired organs were weighed together.

(b) Histopathology

Control and high dose level animals from the main study were examined for histopathology. The brain [medulla/pons, cerebrum and cerebellum], kidney, nerve [sciatic/tibial/sural], spinal cord [cervical/thoracic/lumbar] were examined.

(i) Preservatives: 10% neutral buffered formalin. Eyes were placed in glutaraldehyde/paraformaldehyde initially and then retained in 10% formalin. Lungs and urinary bladder were infused with formalin to insure fixation.

(ii) Processing: After fixation, the selected tissues and organs were routinely processed, embedded in paraffin, cut at a microtome setting of 4 to 7 microns, mounted on glass slides, and stained with H and E.

C. Statistical Analysis

Body weight, body weight change, food consumption, water consumption, hematology and clinical chemistry, urine chemistry parameters, terminal organ and body weights and organ/body weight ratios and organ/brain weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Statistically differences from the control were indicated.

Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, non-parametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly from the control. If a non-parametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if the differences indicated, a summed rank test(Dunn)was used to determine which treatment differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the non-parametric case Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) test was conducted at the 1%, two sided risk level. All other statistical tests were conducted at the 5% and 1%, two sided risk level.

The variances of the two groups were tested for equality using the F test. If the variances were equal, a standard independent two sample t-test was used to determine the equality of means. If the variances differed at the 1% level of significance, Welch's t-test was used to determine equality of means. T-tests were conducted at the 5% and 1%, two-sided risk level.

IV. RESULTS

No test material related deaths occurred. All animals were free of significant pharmacological and toxicological signs throughout the study. Neurobehavorial (Irwin modified screen) responses were not remarkable compared to the controls. Food consumption, water consumption, clinical chemistry, urinary parameters, and organ weights and ratios were not remarkable compared to the respective control values. Gross and histopathology (except for skin lesions) were also not remarkable.

Mean body weight at termination of dosing on Day 11 was decreased by 5% in the male and 2% in the female vs. the respective control values. At termination (Day 29), body weights were decreased by 8% in the male and 6% in the female vs. the respective control

values. These changes were not considered to be of any toxicological importance.

In the high dose level males from days 1 to 11, a decrease [39.5%] in the rate of weight gain occurred (23.9 gm treated vs. 39.5 gm control). No changes were seen in the other dose levels.

In the high dose male recovery animals (to day 29), a decrease in the rate of weight gain [27.8%] occurred (57.8 gm treated vs. 80.1 gm in the control. No changes were seen at the other dose levels.

In the females, mean body weights at all dose levels showed no changes from days 1 to 11. Body weight gain, however, was decreased from 20.4 gm in the control to 16.3 gm in the intermediate dose (20%), a similar [21%] decrease was noted in the high dose.

In the female high dose level recovery animals (to day 29), the rate of weight gain was similar between treated and control groups.

The mean white blood cell count [11.61 x $10^3/\mu l$ (C), 12.65 (Low), 14.84 (Mid), 14.90 (High)] of the test males exhibited a dose related increase compared to the control at the end of the dosing phase which correlated with an increase of 2.5X [4.04 x $10^3/\mu l$ (H) vs. 1.15 x $10^3/\mu l$ in the control, ($p<0.01$)] in the mean absolute neutrophil value of the high dose males. No changes were seen at the end of the recovery period in either the mean WBC or the mean absolute neutrophil count. The mean hematology values of the remaining treatment groups were comparable to the respective control groups. Females were not remarkable.

At the end of the dosing phase, the mean total protein value of the high dose females was decreased [6.0 gm/dl, ($p<0.01$)] compared to the control (6.4); the values were comparable by termination of recovery. This decrease was produced by a decrease in the globulin [2.0, ($p<0.05$) component vs. the control(2.3).

At the termination of dosing, the mean gamma glutamyl transferase value of the high dose females was decreased ($p<0.01$) compared to the control value. The value, however, was comparable, by the termination of the recovery period. This decrease in the enzyme value was not considered to be of any toxicological importance.

The mean organ weight of the treated groups were generally comparable to the mean organ weight of the controls. The few values which were statistically significant ($p<0.05$) different

from the controls were not considered to be biologically significant.

The test material produced skin irritation at the application site in the mid and high dose animals of both sexes as evidenced by focal areas of discoloration and/or scabs. Moderate erythema, edema, eschar and/or tissue damage occurred. Reversible black staining at the application skin site was seen at all dose levels except the control throughout the dosing period. Staining, disappeared during the recovery period. This staining was considered to be due to a chemical reaction and not dermal irritation.

Histological changes consisted of mild to moderate acanthosis [♂:8/10; ♀:3/11] and hyperkeratosis of the epidermis [♂:8/10; ♀:3/11]], exudate formation [♂:8/10; ♀:1/11] and focal epidermal ulcerations [♂:4/10; ♀:1/11]. Most affected animals had an accompanying mild, acute dermatitis [♂:7/10; ♀:1/11]. All recovery animals were not remarkable by Day 25 under gross examination.

Histological changes in the treated skin section from rats in the other dosage groups (including the controls) occurred as single incidences and were similar between the groups.

V. DISCUSSION

Although compliance with GLPs and quality assurance statements were not noted in the compliance statements on p. 2 to 3, Vol 1; however, these statements were included in MRID 452510-01 of Vol II, Appendix P1.

The assay to verify the concentration, stability and homogeneity of the test substance in the carrier was not performed. Since a dose response was seen in body weight gain, these exceptions were not considered to affect the interpretation of the data in this short term study.

The historical data base from male CD® rats, 10 to 12 weeks of age, indicated a normal range of WBC count of 6.0-18.0 x $10^3/\mu l$ [Derelanko, M.J., Toxicologist Pocket Handbook, CRC Press, Boca Raton, 2000]. The dose related increase in the WBC count of males [11.61 x $10^3/\mu l$ (Control), 12.65 x $10^3/\mu l$(Low), 14.84 x $10^3/\mu l$ (Mid), 14.90 x $10^3/\mu l$, $p<0.01$ (High)], therefore, were within the normal range. These changes did not occur in the females.

Although the study was adequate with regard to the methods and procedures, the study did not follow the guideline requirements with regard to the study length and, therefore, was considered to be not-acceptable for a [§ 82-2] Repeated Dose Dermal Toxicity

Study in the rat. Albeit, the endpoints from this study are suitable for use for the short term risk assessment phase.

Appendix 1

Neurobehavioral Study (Modified Irvin Screen)

(from Appendix D, p. 70-71, MRID 452510-01)

1. Home Cage Observations: Before opening the cage, observe the animals for: Posture and Vocalization

2. Handling Observations: Remove the animals from its cage and observe for: Removal/Handling, Salivation, Lacrimation, Chromodacryorrhea

3. Open Field Observations and Reflex Assessments: Place animals on a flat surface and observe for at least 1 minute for:

Gait, Locomotion, Piloerection, Approach, Finger Snap (or Hand Clap), Pupil Response, Abnormal Movements, Tremors, Convulsions, Arousal, Exophthalmia, Tail Pinch, Surface Righting, Visual Placement.

References

Irvin, S., Comprehensive Observational Assessment: Ia: Systemic Quantitative Procedure for Assessing the Behavioral and Physiologic State of the Mouse, *Psychopharmacologia*, **13**, pp. 222-257 (1968).

Moser, V.C., Screening Approaches to Neurotoxicity: A Functional Observational Battery, *Journal of American College of Toxicology*, Mary Ann Liebert, Inc. Publishers, Vol. 8, No. 1, 1989, pp. 85-94.